STUDIES OF SEED INJURY IN CEREALS RESULTING FROM SEED TREATMENT

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STUDIES OF SEED INJURY IN CEREALS RESULTING FROM SEED TREATMENT.

By

J. B. Skaptason

INTRODUCTION.

Historical Review.

Seed treatment for the prevention of bunt, by one method or another, has been practised for nearly three centuries. Before the true nature of the disease was known many of the more intelligent farmers conceived methods of seed treatment on the theory that the seed needed a stimulus to enable it to resist the disease, hence the use of fermenting animal manures. With the idea of the infective nature of smut came the use of toxins. The origin of the use of copper sulphate has been generally credited to Kühn, but he makes no claim to originality and credits it to Prévost, who in turn passes it back to Tessier (30).

Guether suggested the use of formaldehyde as a sterilizer or sterilizing agent in combatting smuts in general and supported his recommendation with experimental evidence (15). Bolley (4) gives the results of laboratory and field experiments in the treatment of seed wheat for the years 1893 to 1896 inclusive. In these experiments he used copper sulphate, mercuric chloride, hot water and formaldehyde. This is the first recorded use of formaldehyde in the

United States as a fungicide for the prevention of bunt. He considered corrosive sublimate superior to all fungicides, with the probable exception of formaldehyde which at the time had received only preliminary tests.

From 1900 to 1903 Farrer (10) carried on extensive seed treatment experiments in Austrailia from which he concluded: "1. Copper sulphate was fairly efficient in killing the spores on the grain, and although it was more injurious to the seed than either formaldehyde or mercuric chloride, it furnished greater protection against reinfestation. 2. Formaldehyde was cheap, effectual and safe, and compared to copper sulphate was non-injurious to the seed, but afforded no protection against reinfestation." Following this and findings of other workers, the use of either copper sulphate or formaldehyde has been determined largely by local practices. More recently however, formaldehyde appears to have been favored and used almost in all countries where seed treatment for bunt is necessary. This is accounted for by its cheapness, ease of handling and its less injurious effects than copper sulphate. Within the last few years these fungicides are being replaced by various mercurial compounds used in dry form and diluted with inert dusts. Due to their costs however, it will probably be sometime before they become generally used.

During the forty years approximately that formaldehyde has been used as a dip or spray, as a gas, Perdrix (24), Thomas (29) or with steam, Melhus (23) reports have differed

radically in the degree of favor with which it has been viewed. Varying degrees of injury have been reported by various workers. Other workers again, principally Stewart and Stephens (29) noted vitality reduction in wheat, oats and barley, but thought the advantages outweighed the injury.

That formaldehyde does cause seed injury is obvious from the results of findings of many workers natably, Hurd (17), Brittlebank (6), Brown (7), Coons and McKinney (8), Cranefield (9) and many others. The importance of this injury becomes at once apparent when one realizes that formaldehyde is being used for smut prevention in all the major wheat growing areas of the world and probably more than any other fungicide in western Canada.

One of the modern trends in plant pathology is a search for chemicals which are efficient fungicides and free from injurious effects. Formaldehyde is a cheap and efficient fungicide but it usually has some injurious effect on the grain. It is being realized now that besides formaldehyde having a direct injurious effect on germination it may occasion certain indirect effects for example in delaying emergence, increasing severity of damage from molds, and rendering plants more susceptible to adverse conditions in the spring. These indirect injurious effects from the formaldehyde treatment of seed may be of equal or greater importance than its direct effects.

Object of investigation.

This investigation has been confined to a study of formaldehyde seed treatment because of the general use of this

fungicide and because of its known injurious effects. The project was undertaken for the purpose of investigating the following aspects of formaldehyde seed injury viz:

1. Factors affecting formaldehyde seed injury.
2. The effect of formaldehyde seed injury on the development of certain diseases.
3. A study of cereal varietal reaction to formaldehyde seed injury.
4. A study of the nature of formaldehyde seed injury with particular reference to diastatic activity.

FACTORS AFFECTING FORMALDEHYDE SEED INJURY.

Literature Review.

A number of factors have been shown to influence the severity of seed injury by formaldehyde. Delay in sowing after treatment and sowing in unduly dry soil were found by Miss Hurd (17) to increase the injurious effects. This author believes that when seeds are treated in formaldehyde and subsequently allowed to dry the polymer paraformaldehyde is deposited on the seed coat with serious eventual consequences. Probably the greatest injury from formaldehyde treatment occurs on grain which has been mechanically injured from machine threshing. Hurd (17) reports twice as much damage in machine threshed grain as hand threshed material, and that

the greatest injury occurs when the seed is broken over the embryo. It has been shown by Hurd (17), Braun (5), Atwood (1) and many others that injury increases proportionately with an increase in the concentration of the formaldehyde. Hurd reports no injury from the recommended 1-320 concentration of formaldehyde or even from 2-320 concentration where treatment consists in immersion of the grain for 10 minutes and for periods up as high as 8 hours. Atwood (1) reports the first decrease in emergence with 20 minutes immersion in formaldehyde of 1-320 concentration. Hurd (17) considers humidity to be the determining factor in seed injury. The formation of paraformaldehyde was dependent on the dryness of the atmosphere. She shows that there was no injury in the damper atmosphere of a 70% humidity and over. Other factors, such as maturity of the grain when harvested, viability of the seed, conditions under which the crop was raised, influence the amount of injury from seed treatment.

Effect of Soil Temperature.

The soil-temperature factor was chosen for study since equipment was available and since it seemed a factor which might be of considerable importance under field conditions. Most of the wheat sown in western Canada is sown in the spring when the soil temperature is relatively low, whereas the coarse grains are sown somewhat later in soil of slightly higher temperature. These studies were made with wheat.

Experimental Methods.

The experiment was conducted in soil temperature tanks in which constant temperatures from 10° to 30° C. were maintained. Soil moisture was kept constant for all temperatures. It was maintained at a minimum for germination since dry soil is known to favor seed injury by formaldehyde. The soil was made up of three parts of Edmonton black soil plus one part of sand, and not sterilized.

Marquis wheat from the 1933 crop was used. The seed was treated by immersion for one-half minute in formaldehyde solution, or in the case of the checks in distilled water, then covered for four hours with bags and dried. Two concentrations of formaldehyde were tested, 1-320 (recommended commonly for the treatment of cereal seeds) and 2-320. The seed was planted immediately. After allowance of sufficient time for the seedlings to emerge counts were made of the percentage of seedling emergence, and by taking the difference between the figures for the treated lots and those of the check the per-cent seed injury was calculated.

Experimental Results.

The results of the soil-temperature experiment are summarized in Table I and Figure 1.

Table I.

Influence of Soil-Temperature on Seed injury by Formaldehyde.

Treatment	Per-cent seedling emergence at different Soil Temperatures °C.				
	10°	15°	20°	25°	30°
Water (Control)	88.0	72.2	63.2	50.1	32.7
Formaldehyde 1-320	75.0	53.2	52.7	45.5	30.7
Formaldehyde 2-320	47.0	43.5	46.7	41.5	26.9

It will be seen from these that seed injury was more pronounced at the lower soil temperatures than at the higher, and especially so at 15°C. This happens to be about the average soil temperature at which much of the wheat is often seeded in the Edmonton district. For instance, the average soil temperature at seeding depth between April 20th and May 10th in 1934 was 13°C. Further tests at low temperatures, however, should be made in order to determine more accurately where the maximum injury occurs. The results so far are in agreement with those of Gassner (13) who states that formaldehyde causes greater damage at the lower temperatures than the higher, while the reverse is true for mercurial dust preparations.

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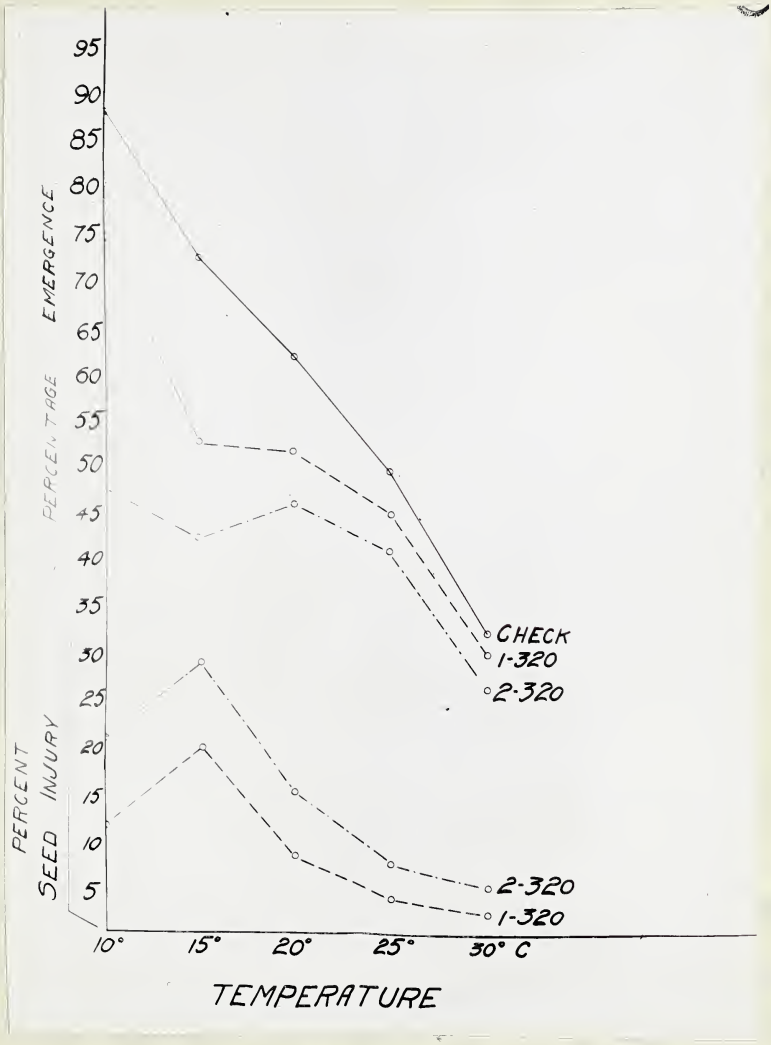


Figure 1.

Effect of soil temperature on seed injury of wheat by formaldehyde.

EFFECT OF CHEMICAL SEED INJURY ON THE DEVELOPMENT OF CERTAIN DISEASES.

Wheat seedlings handicapped in their early stages of growth by various kinds of injury to the seed are rendered more susceptible to invasion from saprophytic and parasitic organisms. The attempted study here is an effort to determine the effect of chemical seed injury. From formaldehyde treatment, on the subsequent disease development from pathogenic organisms common to Alberta soils. The specific investigation was outlined to include various degrees of seed injury caused by different concentrations of formaldehyde and also the severity of damage from two groups of fungi viz: seed-borne and soil-borne organisms.

Literature Review.

Geuther (14) found treatment with mercuric chloride a perfect protective against Penicillium and Fusarium, while formaldehyde promoted the attack of these fungi. The work of Hurd (18) indicates that either chemical injury by $CuSO_4$ or formaldehyde, or mechanical injury render germinating wheat kernels more susceptible to molds. Machacek and Greaney (20) report increased injury from foot-rots when mechanically injured seed was used over that when normal kernels were used. Mead (22) has recently reported that seedlings from seed injured by various agents including rust, drought, frost, sprouting and formaldehyde were more susceptible to attack by Helminthosporium sativum than were seedlings from normal kernels

Influence on Diseases Caused by Seed-Borne Organisms.

In the following experiments Helminthosporium and Fusarium were used as seed-borne organisms. Spore suspensions were prepared and the spores sprayed on to seed which had previously been injured with different concentrations of formaldehyde. The spores of the bunt fungi were shaken up with the seed in envelopes just previous to planting. It was thought that the formaldehyde might exhibit different residual effects on fungus spores which were in contact with the chemical on the seed than those which were not as in the case of Ophiobolus and Pythium.

Formaldehyde seed injury in relation to the severity of foot-rot diseases of wheat caused by Helminthosporium sativum P.K. and B. and Fusarium graminearum Schwabe.

Experimental Methods.

Marquis wheat from the 1933 crop was used throughout for this experiment. Lots of the wheat were treated with formaldehyde of the following concentrations: 1 part of commercial formaldehyde (37%) to 360 parts of water, 1-320, 2-320, and 3-320. The check was treated with distilled water. Treatment consisted of immersion in the formaldehyde solution for

2 minutes followed by a covering of four hours with moist sacks. The seed was then allowed to dry over night. Next morning separate lots were inoculated with spore suspensions of Fusarium graminearum and Helminthosporium sativum as follows: suspensions were made up from 15-day old cultures in sterile water, and sprayed on the seed with an atomizer. The seed was then placed immediately in a desiccator where it was allowed to dry for 24 hours. It was then planted, 25 seeds per pot, in sterilized soil, which consisted of 3 parts Edmonton black soil plus 1 part sand. The experiment was replicated 10 times. The plants were harvested after 21 days, and notes taken on emergence, height and infection rating of individual plants.

Experimental Results.

Data on emergence, height and infection rating are presented in Table II. Percentage emergence is calculated on the basis of the number of surviving plants from uninoculated water treated seed which is taken as 100%. Height figures represent the average height of surviving plants in centimeters. Infection rating is a numerical estimation taking into consideration the percentage of plants which did not emerge and the extent of necrosis of the crown, mesocotyl and primary root system.

TABLE II.

Effect of various Concentrations of Formaldehyde on the Damage to Wheat Seedlings Caused by Seed-borne Organisms.

	Non-Inoculated Seed			Inoculated Seed					
	Check			Helminthosporium			Fusarium		
Formaldehyde Concentration	% Emerg.	Av. Ht.	Av. Inf. rat.	% Emerg.	Av. Ht.	Av. Inf. rat.	% Emerg.	Av. Ht.	Av. Inf. rat.
Water Check	100.0	31.1	0.6	74.8	26.6	47.3	80.3	27.9	22.3
1-360	96.7	29.5	0.4	59.6	24.7	56.3	33.5	20.2	76.1
1-320	90.9	30.6	0.0	67.0	25.9	51.2	48.6	21.2	62.2
2-320	73.9	26.4	0.6	53.6	25.7	53.6	30.5	17.0	73.9
3-320	47.2	23.4	1.0	37.6	22.4	49.5	30.5	11.7	43.2

Emergence.

On taking notes on emergence it was evident that germination was delayed 1 to 1½ days by the 3-320 concentration of formaldehyde in comparison with the check. Intermediate delays were caused by the other concentrations. Emergence results are illustrated in Figure 2. Differences of 10% and greater are considered significant. From the graph it will be observed that for uninoculated seed, emergence decreases uniformly as the formaldehyde concentration increases. For inoculated seed we get a very evident decrease at the 1-360 concentration which is significant in comparison with the water treatment, and in the series inoculated with Fusarium is significantly lower than the 1-320 concentration. In general the data brings out two important points; (1) the excessive damage produced by the 1-360 concentration, (2) that the 1-320 concentration is probably the most favorable one to use in so far as emergence is concerned.

Figure 4, shows the emergence of wheat seedlings just previous to harvesting.

Height of Plants.

The height of plants presented in the above data illustrates how these figures follow somewhat the trend of emergence in that there is a decrease in height even at the 1-360 concentration. The curve in the case of both

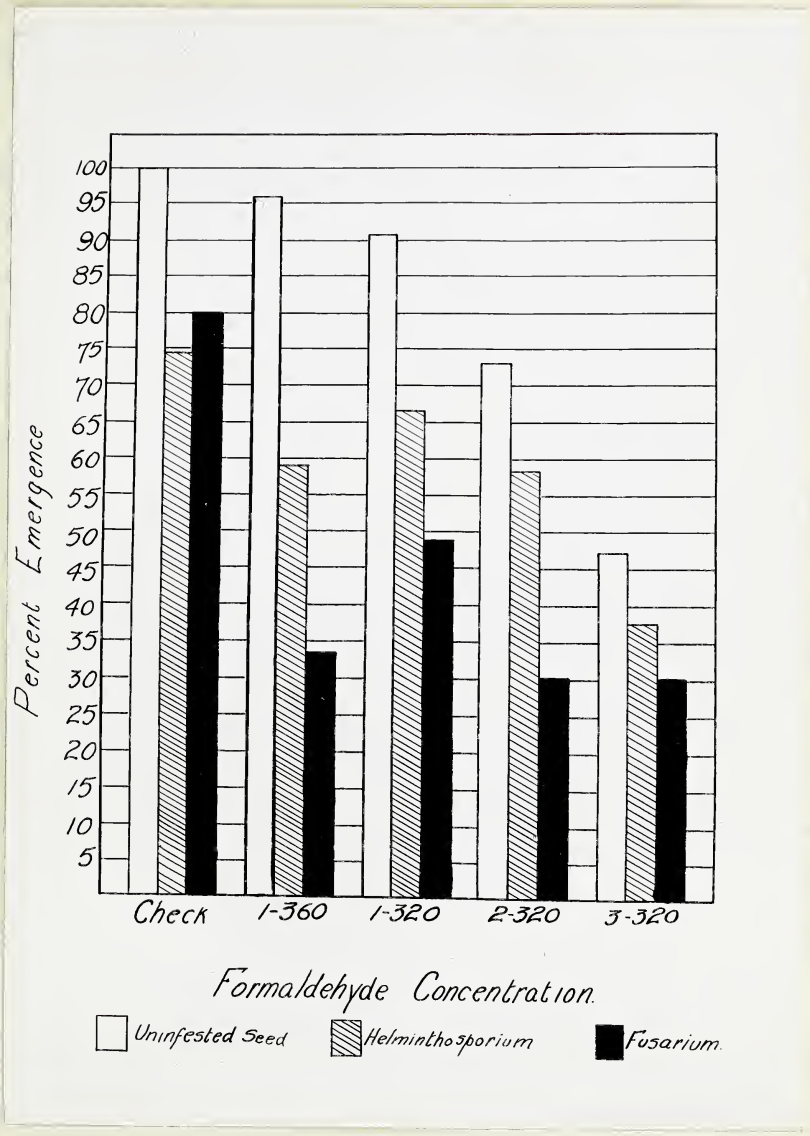


Figure 2.

Effect of formaldehyde seed treatment on the emergence of wheat seedlings from seed inoculated with Helminthosporium sativum and Fusarium graminearum.

organisms rises slightly again at the 1-360 concentration. The table also indicates that Fusarium graminearum caused a greater decrease in the height of plants than Helminthosporium sativum.

Infection Rating.

The strains of Helminthosporium sativum and Fusarium germinearum used were isolated at this laboratory and have proved to be very pathogenic. Careful temperature records were maintained throughout the experiment. Soil temperatures varied between 14°C. and 25°C., averaging 20°C. Air temperatures recorded by a thermograph varied between 15°C. and 32°C., averaging about 21°C.

Infection rating figures illustrated in Figure 3, demonstrate the effect of chemical seed injury on increasing severity of damage from seed-borne organisms. The trend is a marked increase in infection rating on seedlings from formaldehyde injured seed over that on seedlings from normal seed. The 1-320 concentration appears to be the most satisfactory one tested from a standpoint of seed injury and infection rating. Both organisms produce excessive damage on seedlings from seed treated with the 1-360 concentration. The decrease in infection rating from the 3-320 concentration can be partly accounted for by the residual effect of the formaldehyde on the grain at this concentration.

Fusarium produces more pre-emergence blight than does Helminthosporium, and plants failing to emerge were scored as killed: therefore, Fusarium in general produces a higher infection rating than does Helminthosporium.

No statistical analysis was made of the data from results of experiments with these organisms as differences were sufficiently pronounced to interpret without such an analysis.

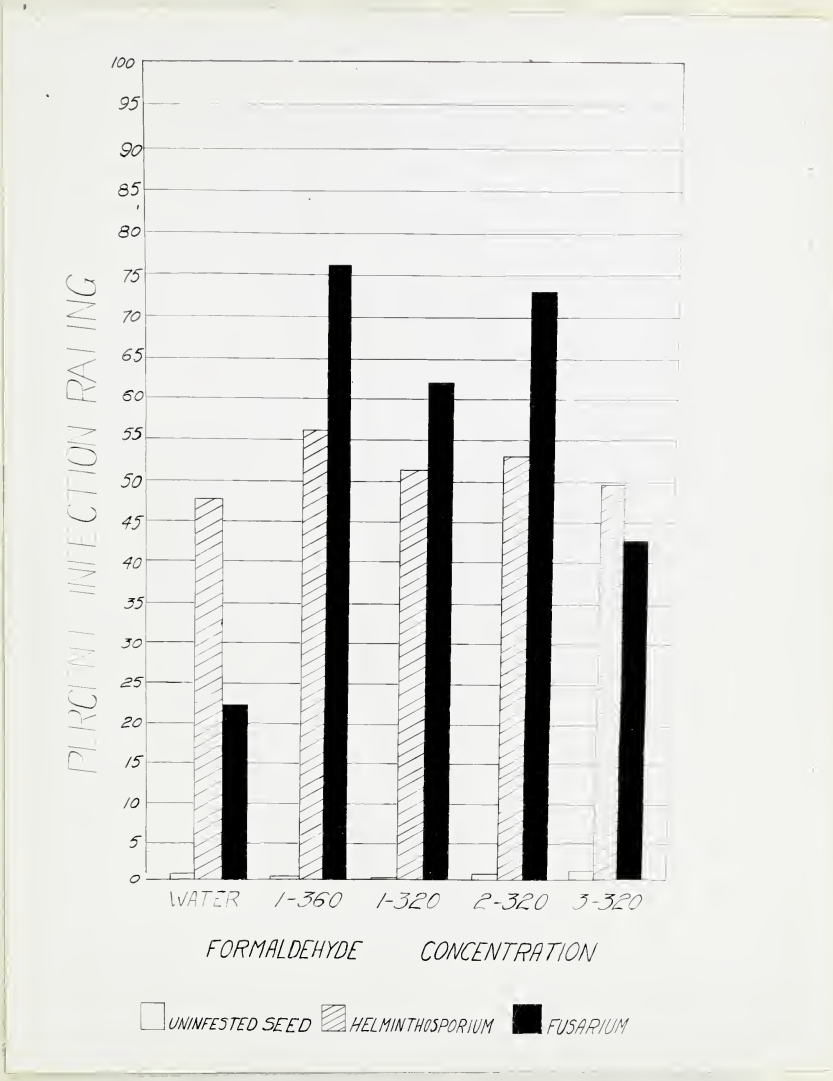
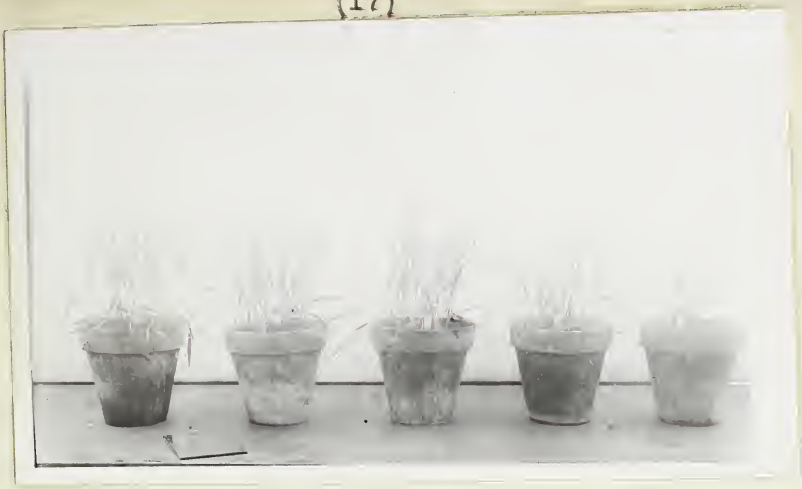


Figure 3.

Effect of formaldehyde seed injury on severity of infection by seed-borne fungi (H. sativum and F. graminearum).



Uninfested soil.

Helminthosporium.

Fusarium.

Figure 4.
Effect of formaldehyde seed injury on the severity of foot-rot diseases of wheat.

Left to right: Check; 1-360; 1-320; 2-320; 3-320.

Effect of Formaldehyde seed injury on infection from bunt fungi.

Formaldehyde is the chief chemical used for the prevention of bunt in wheat. In view of the results obtained with experiments on Helminthosporium sativum and Fusarium graminearum it was thought, however, that formaldehyde might by reason of its seed injury cause an increase in the severity of bunt infection resulting from recontamination of the seed.

Experimental Methods.

Red Bobs 222 a very bunt susceptible variety, was used throughout for this experiment. The experiment was conducted in flats containing unsterilized soil. One hundred kernels were sown in each flat and the experiment was replicated four times. The grain was treated with the various concentrations of formaldehyde by immersion for 1 minute. The treated grain was covered 6 hours and then allowed to air dry for 12 hours. Following this the seed was artifically smutted with a composite sample of Tilletia tritici (Kühn) and T. laevis at the rate of 0.5 grams of smut per 100 grams of grain. The flats were kept in a cold chamber for 10 days where an average soil temperature of 11°C. was maintained. Following this they were removed to one of the colder sections of the greenhouse.

Experimental Results.

The experiment was terminated when the plants had matured sufficiently to take smut notes. Notes were taken on

the number of surviving plants, height and per-cent of smutted plants. Results are presented in Table III:

Table III.

Effect of formaldehyde seed injury on height, emergence and smut infection of Red Bobs Wheat.

Treatment to seed.	No. of surviving plants.	Av. height of plants in inches.	Per-cent bunted plants.
Water	363	24.1	81.0
1-360	361	25.4	72.5
1-320	351	25.0	77.2
2-320	352	24.8	77.3
3-320	275	26.7	56.4
4-320	270	27.4	54.2

Emergence.

From the data in the above table it will be noted that the number of surviving plants decreased with increasing concentration of formaldehyde. Delay in emergence was pronounced in plants from the treated grain. While plants in the water control emerged in five days those from the grain treated with formaldehyde emerged as follows: that treated with the 1-320 concentration on the sixth day, 2-320 on the seventh day, 3-320 and 4-320 on the eighth day. It is to be noted that the plants in the 3-320 and 4-320 were weak and very sickly

at the time of removal from the cold chamber.

Height of plants.

The data in Table III show that plants from water treated seeds are decreased in height due probably to the high smut infection in this treatment. The increase in height of plants from seeds treated with the concentrations 3-320 and 4-320 may be accounted for by the decrease in emergence at these concentrations which would allow for better growth of surviving plants.

Smut Infection.

Treatment with formaldehyde apparently did not predispose Red Bobs wheat to infection by the bunt fungi. The data however are not satisfactory for its detection because of the high percentage of infected plants in the check. There is instead a significant reduction in the severity of infection in the treated grain especially where the 3-320 and 4-320 concentrations were used. While formaldehyde has little protective value after the grain is dry it does have some and this may have obscured any predisposing effects on the plants which the treatment may have had.

The spores of bunt fungi may be more susceptible to the fungicidal effects of the residual formaldehyde or the paraformaldehyde than those of Helminthosporium sativum or Fusarium graminearum. This may account for the positive evidence of predisposition to attack by the two latter fungi and the negative results for the former.

Figure 5 illustrates the effect of formaldehyde seed injury on infection by the bunt fungi. It was observed in scoring plants for smut infection that a higher percentage of sterile

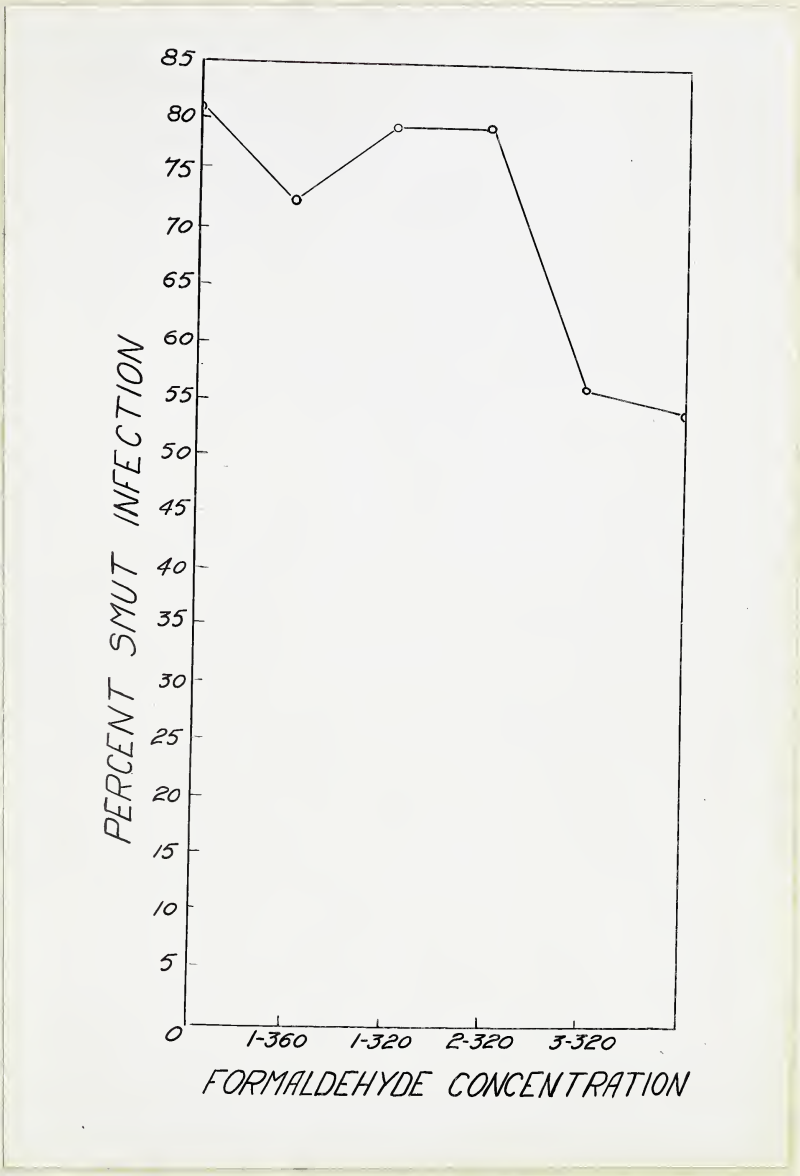


Figure 5.

Effect of formaldehyde seed treatment on smut infection by bunt-fungi (Tilletia tritici and T. laevis.)

plants resulted form treatment with two higher concentrations than from any other concentration. At present sufficient data is not available to discuss this point in detail, but sterility in plants is possible another deleterious effect of formaldehyde which has hither to been unobserved.

Influence on Diseases Caused by Soil-Borne Organisms.

General Methods.

In these experiments inoculum was added to the soil rather than directly to the seed. The inoculum was prepared by growing the organism on a substrate consisting of sterilized Edmonton black soil plus 10% cornmeal and water. This was added to the pots of sterilized soil at the rate of 30 grams per 6 inch pot. The treated seed was then sown directly into the inoculum. Treatment of the seed consisted of immersion of the seed in the different concentrations of formaldehyde for 1 minute, draining, covering for four hours and drying 12 hours at room temperature before planting. The check was treated with distilled water. All experiments were replicated 10 times and the plants harvested after 21 days. The check pots received an equal amount of sterilized soil plus cornmeal in place of the inoculum.

Effect of formaldehyde seed injury on the development of damage from *Ophiobolus graminis* Sacc.

Ophiobolus graminis causes the "take-all" disease which occurs in patches in the field which are more or less

circular in outline. The diseased plants may die at any time from the seedling stage to maturity. The stems, leaves and heads of the plants are bleached; the heads are empty or contain only shrunken grain; the base of the stems and roots are blackened. The strain of Ophiobolus graninis used was isolated in this laboratory from diseased wheat collected at Athabasca, Alberta, in 1930 and has proved to be very pathogenic on wheat. The test was conducted on Marquis wheat from the 1933 crop. The experiment was started in the cold chamber where the pots were kept at an average soil temperature of 13.5° C. following this the pots were removed to the green house where the soil temperature varied between 12° and 20° C. with an average temperature of 17.8° C.

Experimental Results.

Emergence.

It was observed here as in experiments with Helminthosporium sativum and Fusarium graminearum that emergence was delayed somewhat due to the formaldehyde treatment and was more pronounced in infested soil from the combined action of the treatment from formaldehyde and the organism Ophiobolus graminis. Final emergence results are presented in Table IV.

Table IV.

Effect of formaldehyde on emergence in soil infested and uninfested with Ophiobolus graminis.

Seed Treatment	Percentage Emergence	
	Uninfested soil	Infested soil.
Water check	96.6	96.0
1-400	93.6	83.0
1-360	92.0	76.4
1-320	94.8	76.4
2-320	89.2	62.4
3-320	60.0	27.6

These figures show that in uninfested soil, disregarding minor fluctuations, emergence in general is reduced with increasing concentrations of formaldehyde, but does not become significantly reduced until a concentration of 3-320 is reached. However following treatment with 1-400 concentration emergence becomes significantly reduced in infested soil and this reduction is enormously accentuated when more concentrated solutions of formaldehyde as 3-320 are used. These results also indicate that in the presence of infested soil 1-360 concentration is more injurious than the 1-320 concentration.

Height of plants.

In note taking there appeared to be definite differences in height of plants in infested soil between treatments with different formaldehyde concentrations, and it was thought advisable to examine the significance of these differences. Table V shows the height of plants in infested and uninfested soil from seed treated with different concentrations of formaldehyde.

Table V.

Effect of formaldehyde seed injury on the height of Marquis wheat plants in soil infested and uninfested with Ophiobolus graminis.

Formaldehyde Concentration	Height in Centimeters. Uninfested soil	Infested soil.
Water check	42.1	14.2
1-400	42.9	14.6
1-360	42.3	# 11.4
1-320	41.4	13.4
2-320	41.4	## 11.0
3-320	# 37.4	## 9.0

P value exceeds 5% point.
P value exceeds 1% point.

In order to verify the results a thorough examination of the data was essential. Fisher's analysis of variance method was utilized for this purpose. The method of the analysis of the data cannot be given here in detail but will be found in papers by Fisher (11), Fisher and Wishart (12) and Goulden (16). This method of the analysis of randomized pot experiments permits an evaluation of the significance of the experiment as a whole as well as of the individual treatments. The results of the analysis of variance for plant height and formaldehyde concentration in infested soil and uninfested soil are given in Table VI.

Table VI.

Complete analysis of variance for height of plants and formaldehyde concentration in infested and uninfested soil.

Source of variance	Degrees of Freedom	Sums of Squares	Variance	F.	5% Pt.	1% Pt.
Concentration	5	384.84	76.97	13.94	2.30	3.21
Soil treatment	1	25,264.90	25,264.90	4,953.71	3.94	6.90
Concentration x soil treatment.	5	39.09	7.81			
Error	108	596.01	5.52			
Total	119	26,284.94				

To determine if there were any significant differences in concentrations Snedecor's (28) F value was determined and compared with the 5% point. The 5% probability value, which is the value which F must attain to assure a probability

Table IV.

Analysis of variance of weights for twenty-five
plants per treatment combination in pounds
per cultivated unit.

Source of variance	Degrees of freedom	Sums of squares	Mean square	Variance ratio
Replications	4	30.00	7.50	1.00
Soil treatment	1	30,240.10	30,240.10	4,032.01**
Insecticide	3	30.03	10.01	1.33
Soil treatment x Insecticide	3	30.03	10.01	1.33
Error	108	810.00	7.50	
Total	119	30,900.16		

of 20 to 1 against the results obtained being merely due to chance, was used in these studies. The results of the statistical analysis of the data establish that there is a very significant difference in height of plants among the different concentrations of formaldehyde, as the F value greatly exceeds the one per cent point. It will also be observed from the above table that soil treatments are very significant. Referring to the previous table it will be observed that in the uninfested soil only the concentration 3-320 significantly reduces the height of the plants. In the soil infested with Ophiobolus graminis plants treated with 1-360, 2-320 and 3-320 concentrations are significantly reduced in height from the check, but the 1-320 treated plants are not. This is an interesting point and will be discussed again later.

Infection rating.

Per cent infection rating data as determined by scoring individual plants by means of a scale from 0 to 10 (0 clean to 10 killed) are presented in Table VII and illustrated in Figure 6.

Table VII.

Effect of formaldehyde seed injury on the severity of infection by Ophiobolus graminis.

Treatment to seed.	Per-cent infection rating.	
	Uninfested soil	Infested soil
Water check	0	77.9
1-400	0	# 87.0
1-360	0	# 91.0
1-320	0	89.0
2-320	0	92.5
3-320	0	97.0

P value exceeds 5% point.

In Table VIII is presented the complete analysis of variance for per-cent infection rating and seed treatment using Ophiobolus graminis.

Table VIII.

Complete analysis of variance for per-cent infection rating and seed treatment for Ophiobolus graminis.

Source of variance.	Degrees of Freedom.	Sums of Squares.	Variance.
Concentration	5	217.96	43.59
Error	54	3,230.26	59.82
Total	59	3,448.22	

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Table [illegible].

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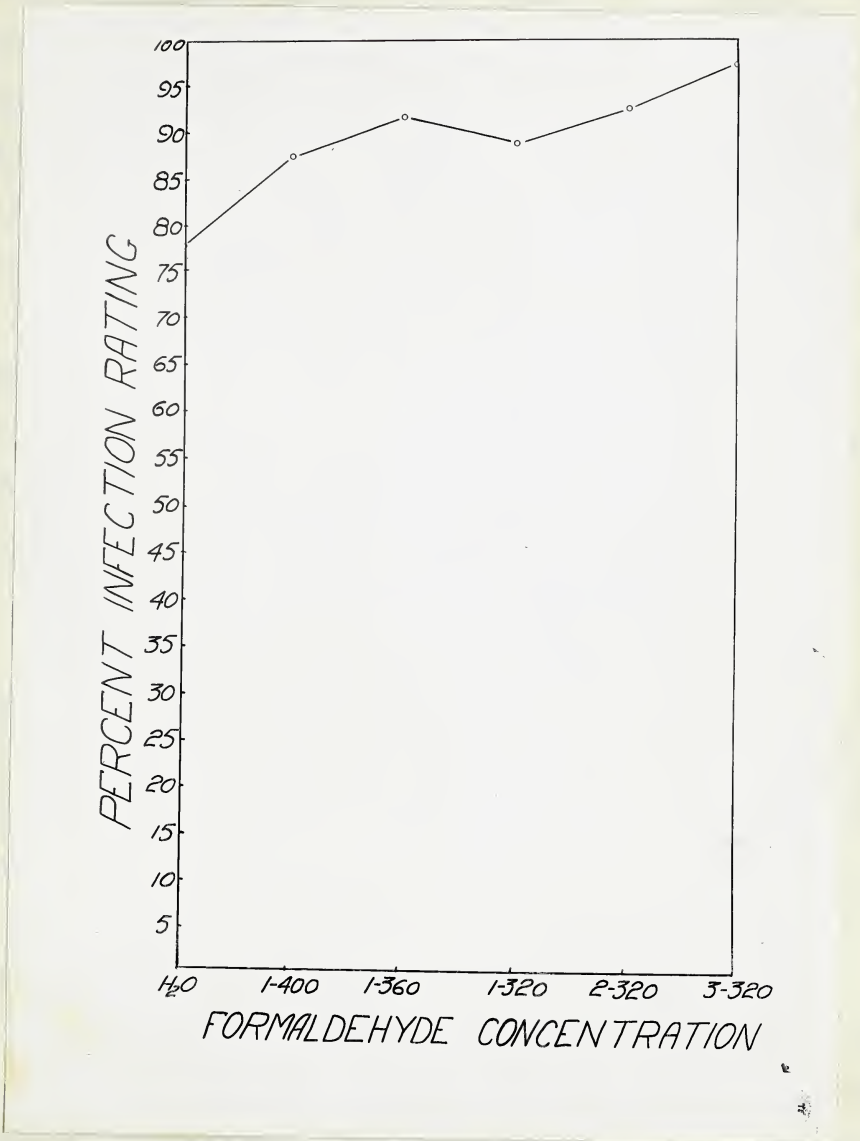

Figure 6.

Effect of formaldehyde seed treatment on the severity of seedling attack by soil-borne inoculum of Ophiobolus graminis.

In the above table we note that variance due to error is larger than the variance due to concentrations. Therefore we may say that the concentrations do not differ significantly among themselves. This does not necessarily mean, however, that differences between any pair of treatments is not significant, and it will be seen from data in the previous table that all concentrations produce a significant increase in infection rating in comparison with the water treated seed. The treatment with the 3-320 concentration is the only one showing an increase in infection rating which is significant over any other concentration. Therefore the other four concentrations may be grouped as one in so far as infection rating is concerned.

Effect of Formaldehyde Seed Injury on the Development of Damage from *Pythium* sp.

Experimental Methods.

The strain of Pythium used in this experiment was isolated from wheat roots sent in from Ashmont, Alberta in 1934. This strain designated P_3 has been tested and found to be fairly pathogenic on wheat seedlings, producing the symptoms of browning root-rot disease.

Inoculum in this experiment was produced by growing the fungus on Edmonton black soil plus 10% cornmeal. This added to 6 inch pots of sterilized soil at the rate of 50 grams per pot seemed to give satisfactory infection. The check or uninfested pots received 50 grams of sterilized soil plus 10% cornmeal in place of the inoculum. The soil temperature throught the experiment varied from 13^0 to 19^0 C. with an average

of approximately 15.9°C. The test was conducted on Red Bobs wheat from the 1934 crop.

Experimental Results.

Emergence.

Emergence delay of plants from formaldehyde treated seed in both the infested and uninfested soil was particularily striking. Daily counts were made in order to follow these trends in emergence delay and particularily to observe the delay caused by intermediate concentrations. In Table IX are shown the daily emergence counts expressed in per-cent. The data show that the final emergence in infested soil is not a great deal different than the final emergence in uninfested soil. It appears, however, that delay in emergence in the different formaldehyde concentrations behaves differently in the infested soil and in uninfested soil.

Table IX.

Daily emergence counts of plants from formaldehyde treated seed in soils infested and uninfested with Pythium.

Formald. Conc.	Daily percentage emergence.											
	Uninfested soil						Infested soil.					
Days	5th	6th	7th	8th	9th	Final	6th	7th	8th	9th	10th	Fin.
Check	12.4	24.4	57.6	76.4	84.0	92.8	9.2	21.2	54.8	76.8	88.4	93.6
1-400	5.6	15.6	47.2	62.4	71.2	91.2	2.0	6.4	34.0	57.2	75.6	92.8
1-360	8.8	18.8	66.8	82.0	86.0	92.0	0	2.4	22.4	44.0	65.6	91.6
1-320	2.4	7.6	41.6	66.0	69.3	89.6	0	5.2	34.0	57.2	69.6	92.5
2-320	4.7	13.2	43.6	70.8	76.4	87.6	0	4.0	22.4	33.6	51.2	79.2
3-320	1.8	5.2	36.0	54.8	61.2	80.0	0	000	13.6	30.4	44.4	64.8

| | | |

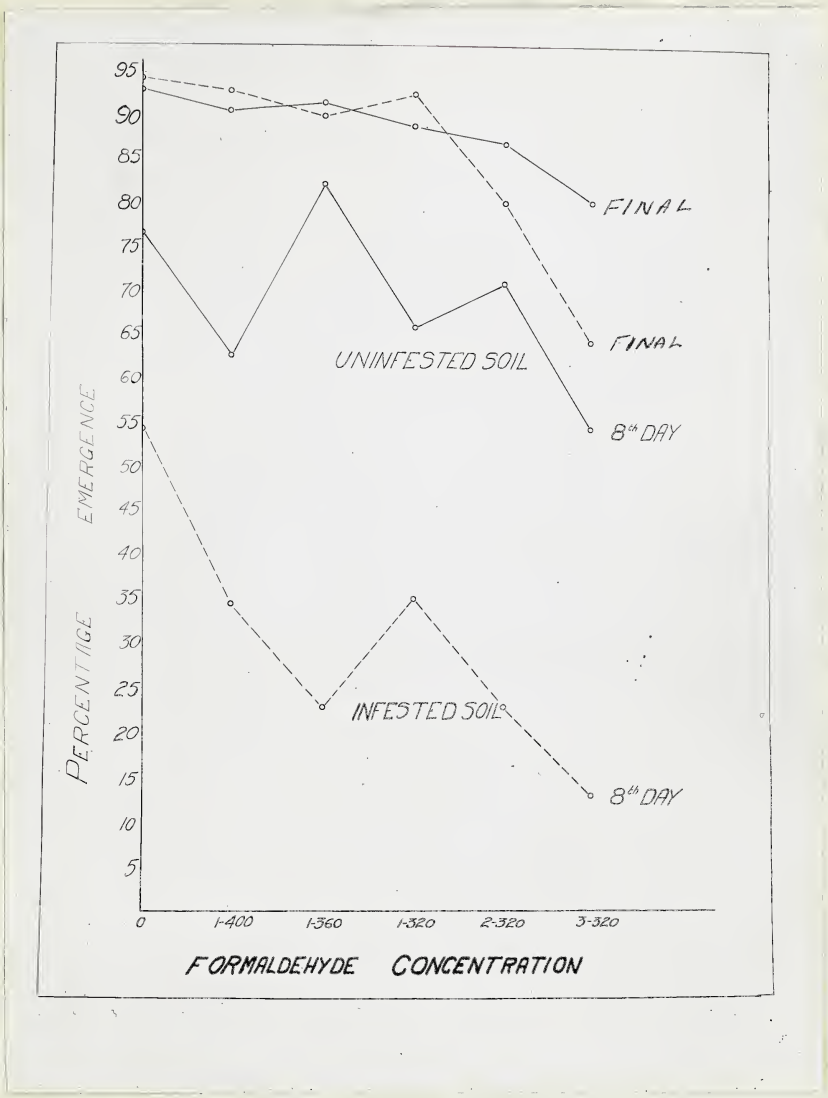

Figure 7.

Effect of formaldehyde seed treatment on emergence of wheat seedlings in soil infested and uninfested with Pythium inoculum, showing final emergence and emergence on the eighth day. Broken line-: infested soil, Solid line-: uninfested soil.

It will be noticed from the data in Table IX, that first emergence occurred in all the treatments in the uninfested soil on the 5th day, whereas no emergence had occurred at all in the infested soil on the 5th day. On the 6th day in the infested soil there was 9% emergence of plants from water treated seed but practically none from the formaldehyde treated seed. The 3-320 concentration delayed emergence 3 days in the infested soil. To illustrate how delayed emergence operates in these intermediate concentrations a graph was prepared, Figure 7, showing the final emergence and the emergence on 8th day in infested and uninfested soils. It is interesting to observe in the uninfested soil how emergence drops at the 1-400 concentration and arises again at the 1-360 and then gradually falls off again, with increase in concentration. In the infested soil there is a steady drop from the water treated seed to the 1-360 concentration when it suddenly rises again at 1-320 and then gradually drops off. This will be discussed further under diastatic activity.

The height of plants under these different seed and soil treatments exhibited unusual differences and it was thought that a more detailed analysis of the data might be valuable in determining their significance. Height of plants in centimeters is presented in Table X and illustrated in Figure 8.

Table X.

Effect of formaldehyde concentrations on the height of plants grown in soil infested and uninfested with Pythium Inoculum

Formaldehyde Concentration.	Height in Centimetres	
	Uninfested soil	Infested soil.
Water check	33.5	14.9
1-400	31.4	# 12.1
1-360	35.1	10.3
1-320	33.1	12.2
2-320	32.6	9.1
3-320	31.5	7.5

To be significant the difference between means must exceed $2 \times \sqrt{2} \times$ S.E. = 2.40.

A complete analysis of variance for plant height and formaldehyde concentration in uninfested and infested soil is presented in the following table.

Table XI.

Complete analysis of variance for plant height and formaldehyde concentration in infested and uninfested soils.

Source of variance	Degrees of freedom	Sums of squares	Variance	F	5% Pt.
Seed treat.	5	233.60	46.72	6.41	2.30
Soil treat.	1	14,546.43	14,546.43		
Seed treat. x Soil treat.	5	187.60	37.52	5.12	2.30
Error	108	787.61	7.28		
Total	119	15,755.24			

Baby class	[illegible]	[illegible]
I-Std.	[illegible]	[illegible]
II-Std.	[illegible]	[illegible]
III-Std.	[illegible]	[illegible]
IV-Std.	[illegible]	[illegible]
V-Std.	[illegible]	[illegible]

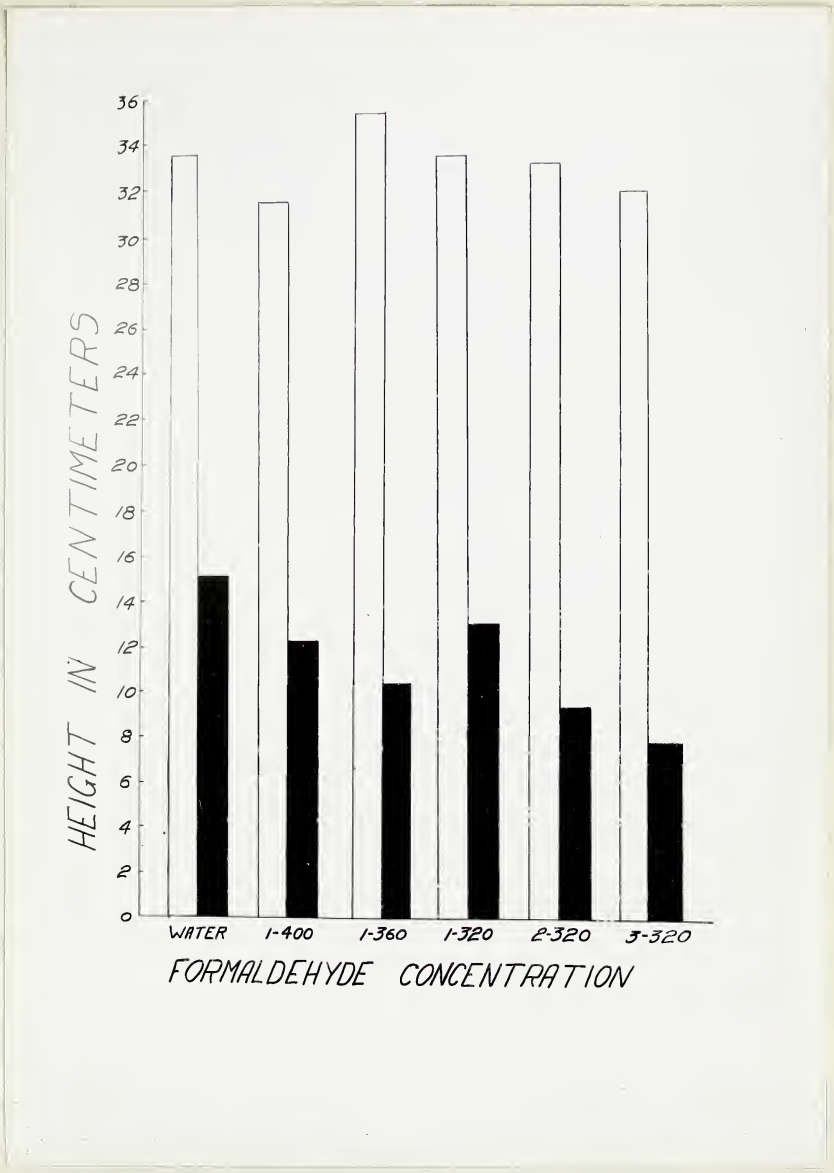

Figure 8.

Effect of formaldehyde seed treatment on the height of plants in soil infested and uninfested with Pythium sp
White pillar--uninfested soil.
Black pillar--infested soil.

Examining the above table we observe that for concentrations the F value is larger than the 1% point therefore the concentrations differ significantly among themselves. Treatment of the soil constitutes the largest variance and is very significant as shown particularily by the previous table. The above analysis also shows that interaction between soil treatments and seed treatment is significant. This is interpreted to mean that the height of plants under different formaldehyde concentrations is affected differently in infested soil than in uninfested soil. In this case it is apparently due to the reversal of the height figures in the 1-400 and 1-360 formaldehyde concentrations in the infested and uninfested soils. This difference in the hieght of plants is illustrated in the accompanying photograph, which was taken at the time of harvesting, Figure 9.

Referring to Table X further and taking 2.40 centimetres as the minimum significant difference between the means of treatments we observe in the uninfested soil that none of the concentrations significantly reduce the height of plants in comparison to the water treated check. There is a significant reduction in the 1-400 treatment in comparison to the 1-360 treatment. However in infested soil all concentrations are significantly reduced in comparison with the water treated check, and from the data in the table the treatment from the 1-360 concentration is more injurious than 1-400 concentration so far as the height of plants is concerned in infested soil. Also 1-360 concentration is more injurious than the 1-320. This may be directly traceable to the severity of infection which is significantly higher following treatment with the 1-360 concentration as compared with the 1-320 concentration.

Figure 9.

Effect of formaldehyde seed treatment on severity of seedling attack by Pythium sp. Upper-uninfested soil. Lower-infested soil. Left to right: water; 1-400;1-360; 1-320; 2-320; 3-320.

Infection rating.

Due to the lack of definite foot-rot symptoms and blighting it was much more difficult to score the plants in this experiment than those infected with Ophiobolus graminis. However, plants were scored on a scale from 0 to 10 and the infection rating was expressed in per-cent. Plants were just beginning to show symptoms of browning at the time of harvesting. One of the striking features of the plants from seed treated with formaldehyde in infested soil was the complete lack of primary roots in all concentrations with the probable exception of 1-320 where browning symptoms were least marked. This complete rotting of primary roots is shown in Figure 9, of plants grown in infested soil and photographed at the time of harvesting.

The data on per-cent infection rating of plants from treatments with the different concentrations of formaldehyde are presented in Table XII and illustrated in Figure 10.

Table XII.

Effect of formaldehyde seed injury on per-cent infection resulting from Pythium sp.

Formaldehyde Concentration.	Per-cent infection rating.	
	Uninfested soil	Infested soil.
Water check	0	63.7
1-400	0	# 72.9
1-360	0	75.6
1-320	0	69.9
2-320	0	77.5
3-320	0	83.7

To be significant the difference between the means of seed treatments must exceed 2 x 2 x 2.64 = 7.44.

The data were analyzed by Fisher's analysis of variance and the Standard Error was determined by taking the square root of the variance due to error. From this the minimum significant difference was determined by the formula $2 \times \sqrt{2} \times$ S.E. The complete analysis of variance is given in Table XIII.

Table XIII.

Complete analysis of variance for per-cent infection rating and formaldehyde concentration for Pythium.

Source of variance.	Degrees of freedom.	Sums of Squares.	Variance.
Formaldehyde Concentration	5	262.70	52.54
Error	54	3,787.95	67.04
Total	59	4,050.65	

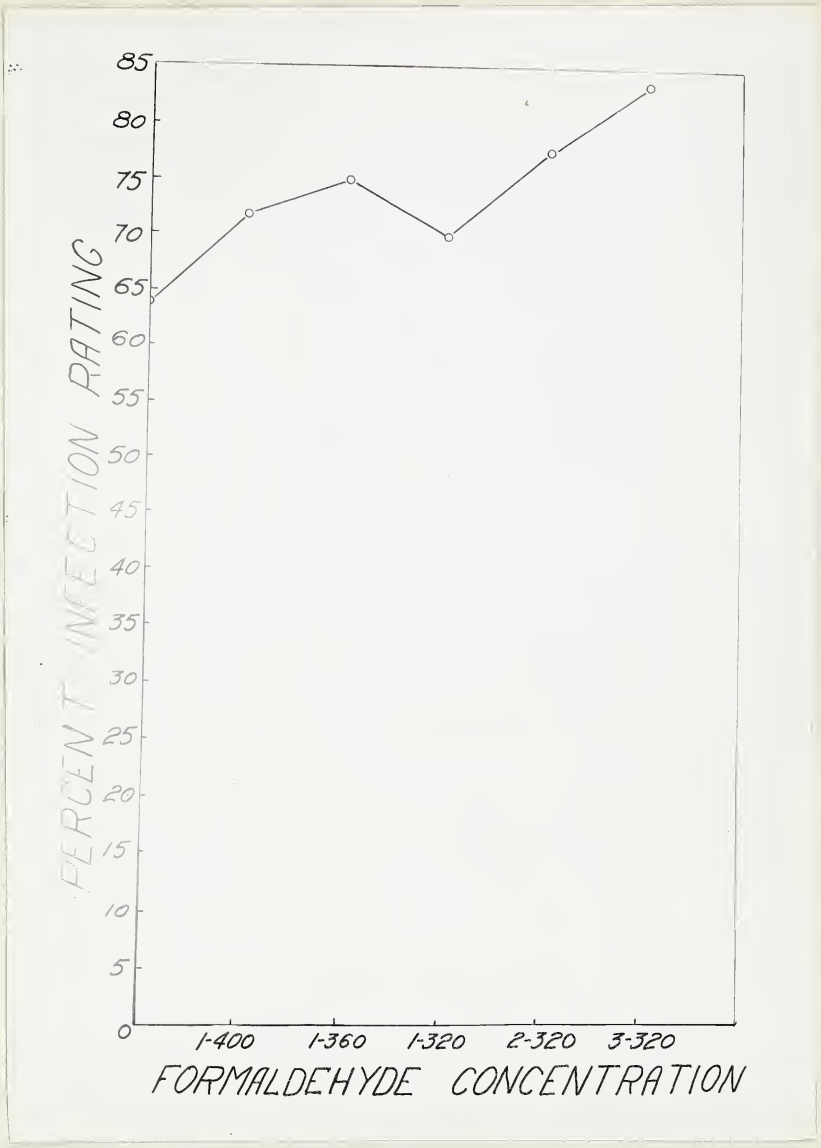

Figure 10.

Effect of formaldehyde seed treatment on the severity of infection by Pythium sp.

The statistical analysis presented above shows the variance due to error to be larger than the variance due to concentrations. Hence the concentrations taken as a whole do not differ significantly among themselves. However referring to the data in the previous table and taking the minimum significant difference as 7.44 it will be observed that all concentrations of formaldehyde produce a significant increase in infection rating over that produced in the water treated seed. As in the experiment with Ophiobolus inoculum the 3-320 formaldehyde concentration is the only one producing a significant increase in per cent infection rating over any other concentration used. Plants from seed treated with the 1-320 concentration of formaldehyde developed the lowest infection rating in the infested soil.

Discussion.

The results obtained with the bunt fungi will be excluded from this general discussion as they behave differently from any of the other organisms studied for reasons stated previously.

In experiments with soil borne inoculum the infection rating increases with the increase in concentration of the formaldehyde. But in seed borne inoculum the infection rating in general increases up to 2-320 and falls off markedly at 3-320. This would seem to indicate that at this concentration the formaldehyde has a fungicidal effect in the case of the seed borne inoculum.

In general the fungi studied produce a significant increase in infection rating in plants from formaldehyde treated seed in comparison with those from water treated seed. Using soil borne inoculum there is no significant difference between the concentrations 1-400, 1-360, 1-320 and 2-320 in infection rating produced, 3-320 showing the first significant increase over other concentrations in this respect. Therefore for the concentrations studied there appears to be no fungicidal effect from the seed treatment. From the data presented it appears in general that 1-320 is the most satisfactory concentration to use as it tends to be least injurious from the combined action of the seed injury and damage from the organism. This may be due to either of two causes, either it produces less injury to the seed or it may possess its greatest fungicidal effedts at this concentration. The 1-360 concentration appears to be the least desirable from this standpoint as it consistently resulted in most damage in the case of soil-borne and seed-borne inoculum. In per cent the actual difference between the two concentrations 1-360 and 1-320 is only 0.01% which, under seed treatment methods employed by farmers is a very narrow range and a slight error in preparing the formaldehyde solution may lead to a very significant increase in the infection from pathogenic organisms.

The practical significance of this increase in infection rating of foot and root-rotting organisms resulting from formaldehyde seed injury is at once evident in a province such as Alberta where the foot-rot and root-rot diseases of wheat are major problems.

REACTION OF CEREAL CROPS AND CEREAL VARIETIES TO FORMALDEHYDE

Reaction of Cereal Crops

It has long been a belief amongst agricultural workers and farmers that barley is more susceptible to injury from fungicides than is wheat. Hurd (17) in this regard concluded that barley is very much more susceptible than wheat to injury from copper sulphate. She concluded that this difference in injury was due to the barley hull being ruptured on each kernel where it was broken from the rachis.

In order to study the relative reaction of different ce reals to formaldehyde single varieties of wheat, oats and barley and also the averages of several varieties were compared.

Experimental Methods.

The grain to be tested was obtained from the Cereal Divison of the Department of Field Crops. It had all been grown under similar conditions in varietal trial plots and had all been threshed and handled in the same way. The experiment during 1933 and 1934 was conducted in 5 row plots, 1 rod long, spaced 1 foot apart and replicated 4 times. Emergence counts and yield determinations were made on the center rows. The grain was treated by immersion in the required formaldehyde concentration for 15 seconds, covered 4 hours and then allowed to dry thoroughly before counting and seeding.

Experimental Results.

In studying reactions of single varieties of wheat, oats, and barley to formaldehyde the varieties Marquis, Victory, and O.A.C. 21 respectively were used. Results of field emergence counts for the year 1933 are presented in Table XVI and illustrated in Figure 11.

TABLE XVI.

Seed injury of wheat, oats and barley resulting from formaldehyde treatment in 1933.

	Per-cent Emergence.		
Formaldehyde Concentration	W H E A T (Marquis)	O A T S (Victory)	B A R L E Y (O.A.C. 21.)
Check	65.5 ± 2.18	69.6 ± 1.76	67.2 ± 1.35
1.-320	# 58.3 ± 1.93	66.4 ± 1.67	70.0 ± 1.40
2-320	32.3 ± 1.07	# 62.1 ± 1.57	#64.0 ± 1.28
4-320	24.6 ± 0.81	60.9 ± 1.54	46.0 ± 0.92
6-320	10.1 ± 0.33	54.7 ± 1.38	23.1 ± 0.46
8-320	6.7 ± 0.22	50.5 ± 1.27	15.1 ± 0.30
	S.E. ± 3.33	S.E. ± 2.53	S.E. ± 2.01

Figures to be significant must be greater than 2 x standard error (S.E.).

The emergence data in the above table with accompanying standard errors calculated by Hayes and Garber's method show that treatment of wheat with 1-320 formaldehyde caused a significant decrease in emergence. In oats and barley the first significant decrease occurred when the 2-320 concentration was used.

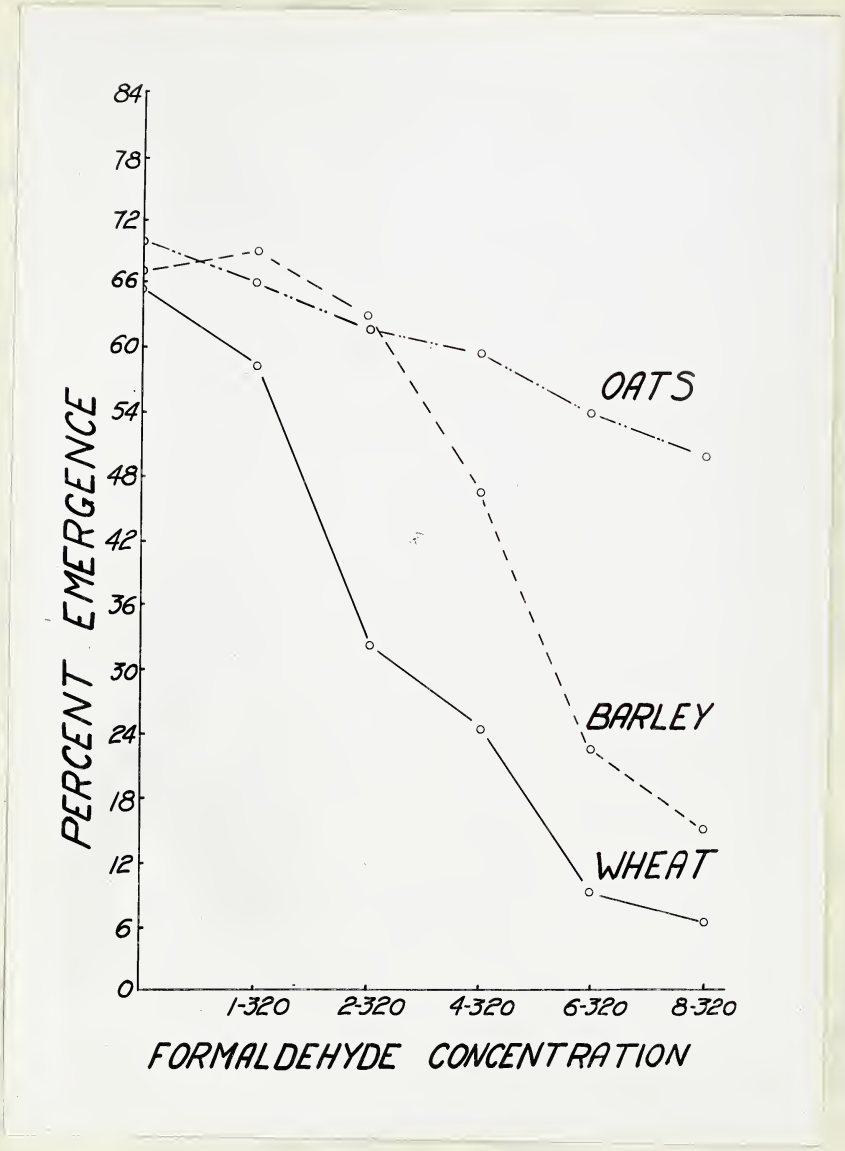

Figure 11.

Reaction of cereal crops to formaldehyde in 1933.

It will be observed from Figure 11, that wheat is most injured, barley the next and oats the least injured.

Experimental results for 1934 are presented in Table XVII, and illustrated in Figure 12.

An examination of Figure 12, shows that in general wheat tends to be more injured than either barley or oats, and that oats are least injured. It is to be observed here that oats are more injured by formaldehyde than they were the year previous. This slight discrepancy in the results for the two years illustrated the extent to which experiments of this nature are subject to seasonal variation. This may be caused by seasonal conditions under which varieties mature, stage of maturity at the time of harvesting, and slight uncontrollable variations in treatment from season to season.

Table XVII.

Seed injury of wheat, oats and barley resulting from formaldehyde treatment in 1934.

Formaldehyde Concentration	Per-cent Emergence		
	W H E A T (Marquis)	O A T S (Victory)	B A R L E Y (O.A.C. 21.)
Check	70.5 ± 0.39	74.9 ± 1.43	83.4 ± 1.21
1-320	# 60.1 ± 0.33	# 62.5 ± 1.20	#73.6 ± 1.06
2-320	42.9 ± 0.24	53.1 ± 1.01	53.1 ± 0.77
4-320	16.1 ± 0.09	39.8 ± 0.76	21.5 ± 0.31
6-320	11.2 ± 0.06	28.2 ± 0.54	15.9 ± 0.23
8-320	3.5 ± 0.02	16.2 ± 0.31	4.4 ± 0.03
	S.E. ± 0.56	S.E. ± 1.92	S.E. ± 1.45

To be significant the difference between the means of treatments must exceed 2 x standard error(S.E.)

Table [illegible].

Head injury or death, data and leading causes from investigations reported in [illegible].

Investigation	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Fatalities					
Check	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1-2[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2-2[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1-3[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2-3[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1-1[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

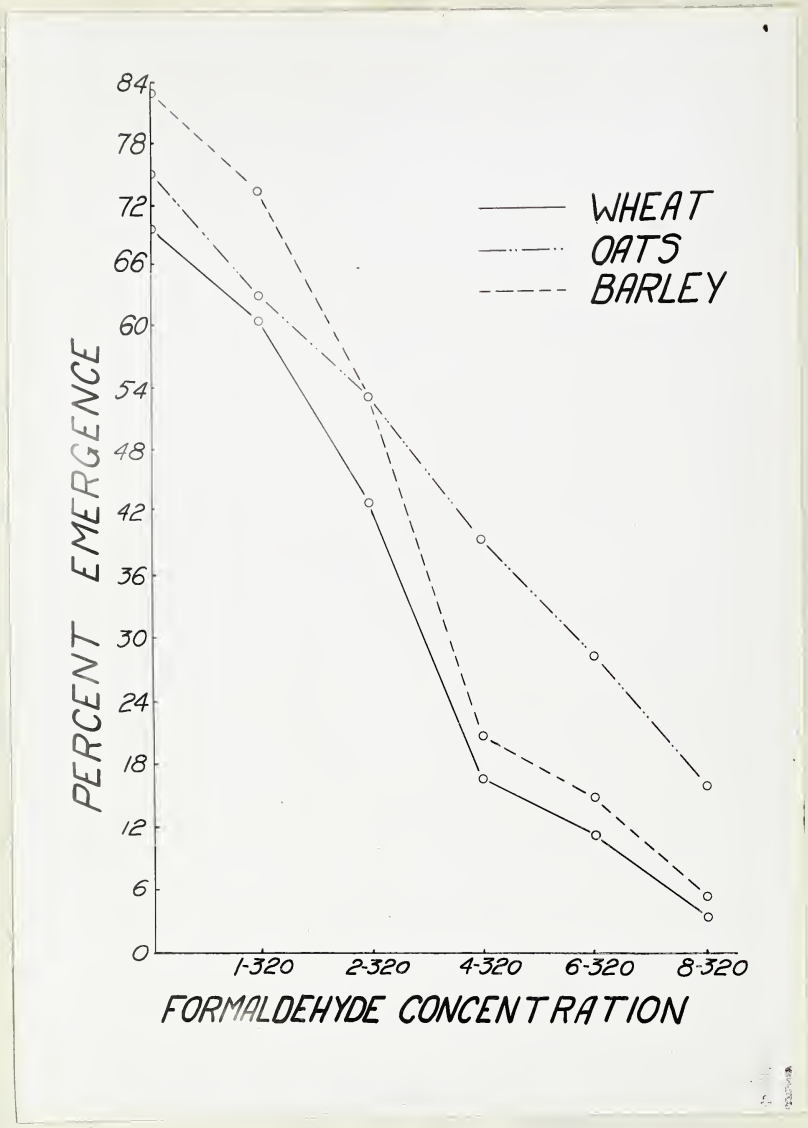

Figure 12.

Reaction of cereal crops to formaldehyde in 1934.

The data in Table XVII, show that emergence in wheat, oats and barley is significantly reduced by a 1-320 concentration of formaldehyde. It will be observed that the S.E. for the experiment in 1934 was a great deal lower than in 1933, this probably being due principally to the greater uniformity of the soil in 1934. Our 1933 plots were contaminated with patches of couch grass and also were damaged somewhat by gophers and wireworms.

The per-cent seed injury calculated on the basis of the decrease in emergence resulting from treatment with a 4-320 concentration of formaldehyde from that of water treated seed was determined for five varieties within each crop. The figures were then averaged to obtain a representative figure for per-cent seed injury by formaldehyde for all varieties of wheat, oats and barley over a two year period. The figures are presented in Table XVIII.

Table XVIII.

Average per-cent seed injury by formaldehyde in five varieties of wheat, oats and barley for 1933 and 1934.

Cereal	Average per-cent seed injury.
W h e a t	35.87
B a r l e y	10.87
O a t s	7.93

Table [illegible].

Average per-unit wood injury to [illegible] tree varieties at Wenatchee, Wash. and Yakima for 1923 and 1924.

Insect	Average per-unit wood injury.
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]

These results show in general that over a period of two years the order of decreasing formaldehyde seed injury in cereals is wheat, barley and oats. The presence of the hull on the barley and oat grains probably affords the protection which makes them more resistant than wheat to the harmful effects of treatment by formaldehyde. The marked resistance of oat kernels may be due to the loose attachment of the lemma and palea to the caryopsis thereby forming an air pocket. The hairy covering on the caryopsis may also help in resisting the penetration of formaldehyde.

Reaction of Cereal Varieties.

Varietal differences of barley in susceptibility to injury by formaldehyde, particularily as indicated by yield data, were demonstrated by Tisdale et al (32). They concluded that different modifications of the formaldehyde treatment would be necessary for different varieties of barley if seed injury was to be avoided.

Experimental Results for 1933.

In studying varietal susceptibility to seed injury by formaldehyde the concentration 2-320 and 4-320 were used. Differences exhibited by emergence were correlated with differences in yield. Figures for emergence only are presented in Table XIX.

Table XIX.

Effect of various concentrations of formaldehyde on the germination of different varieties of cereals.

	Per-cent Emergence.		
Variety	Check	2-320	4-320
Marquis	65.5	32.3	24.6
Reward		21.9	17.6
Garnet		35.6	8.4
Red Bobs		27.1	23.0
Caesium		40.4	27.2
Victory	69.6	62.1	60.9
Banner		68.4	63.0
White Cross		62.6	55.6
Gopher		70.0	59.0
Leader		68.4	67.7
O.A.C. 21	67.2	64.0	46.0
Trebi		65.1	50.8
Hannchen		69.0	70.7
Canadian Thorpe		56.7	60.4
Glabron		59.4	59.5

It will be observed that distinct differences occur between the different varieties of wheat, Garnet according to these figures, showing the greatest susceptibility to seed injury, and Red Bobs 222 the least. Similar differences,

Table [illegible]

Study of various characteristics of [illegible]
in the production of different varieties of [illegible]

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]

though somewhat less pronounced, are shown between the varieties of oats and barley. In oats the variety Leader proved the most resistant of those tested. Varieties like Glabron and Hannchen appear to be very little affected by a 4-320 concentration of formaldehyde, and therefore appear to be very much more resistant to injury than O.A.C. 21 and Trebi. Since all factors such as soil temperature, air temperature, soil moisture and treatment were relatively constant and the only variable being variety, such differences as are observed in the data of the above table must be attributed to varietal reaction.

Experimental Results for 1934.

The results of wheat variety studies made in 1934 are presented in Table XX.

Table XX.

Effect of various concentrations of formaldehyde on the germination of different varieties of wheat.

Variety	Per-cent Emergence.			
	Check	2-320	4-320	8-320
Red Bobs	89.5	87.4	62.1	45.2
Marquis	81.3	75.2	46.3	23.4
Reward	75.4	72.3	35.2	17.5
Garnet	85.2	43.5	22.5	7.6

The figures in the above table show differences in wheat varietal reaction of wheat to formaldehyde, and follow

the general trend obtained the year previous. Red Bobs 222 is the most resistant to seed injury and Garnet the most susceptible with Marquis and Reward intermediate, Marquis is slightly more resistant than Reward.

Results of studies conducted on oats and barley varieties are presented in Table XXI and XXII.

Table XXI.

Effect of various concentration of formaldehyde on the germination of different varieties of oats.

Variety	Per-cent emergence		
	Check	2-320	4-320
Victory	74.9 ±1.32	53.1 ±0.93	39.8 ±0.70
Banner	66.7 ±1.18	50.0 ±0.88	29.6 ±0.52
White Cross	72.6 ±1.28	25.2 ±0.44	15.5 ±0.27
Gopher	72.5 ±1.28	43.1 ±0.76	23.7 ±0.41
Leader	66.9 ±1.18	53.7 ±0.95	33.0 ±0.58

Table XXII.

Effect of various concentrations of formaldehyde on the germination of different varieties of barley.

Variety	Per-cent emergence		
	Check	2-320	4-320
O.A.C.	83.4 ± 2.05	53.1 ± 1.31	21.5 ±0.53
Trebi	73.5 ± 1.81	48.0 ± 1.18	12.6 ± 0.31
Hannchen	81.5 ± 2.01	48.0 ± 1.19	23.5 ±0.58
Can. Thorpe	82.5 ± 2.03	64.4 ± 1.59	20.6 ± 0.50
Glabron	73.1 ± 1.81	57.5 ± 1.42	15.7 ±0.38

Table [illegible].

Effect of various concentrations of [illegible]
on the germination of different varieties of [illegible].

Variety	Check	[illegible]	[illegible]	[illegible]
Victory	[illegible]	[illegible]	[illegible]	[illegible]
Dunbar	[illegible]	[illegible]	[illegible]	[illegible]
Hollis Dwarf	[illegible]	[illegible]	[illegible]	[illegible]
Golden	[illegible]	[illegible]	[illegible]	[illegible]
Climax	[illegible]	[illegible]	[illegible]	[illegible]

Table [illegible].

Effect of various concentrations of [illegible]
on the germination of different varieties of [illegible].

Variety	Check	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cowpea	[illegible]	[illegible]	[illegible]	[illegible]
Soybean	[illegible]	[illegible]	[illegible]	[illegible]
Cow Itaipe	[illegible]	[illegible]	[illegible]	[illegible]
Blackeye	[illegible]	[illegible]	[illegible]	[illegible]

It will be observed that distinct differences occur among the different varieties. In the oat varieties taking the 5% point as the level of significance and the difference between the check and 2-320 it will be observed that all varieties are significantly reduced in emergence, with the probable exception of Leader, which is just on the border line. White Cross is most injured. The three remaining varieties while showing a significant decrease from the check do not exhibit sufficient differences among themselves to warrant placing them in any order of injury. At 4-320 Gopher is significantly injured over Banner and Victory, but the latter two do not show a significant difference one over the other. So probably the final order of placing in regard to increasing seed injury would be Leader, Victory and Banner the same, Gopher and then White Cross.

In barley, using the 2-320 concentration of formaldehyde for testing varietal reaction, and the 5% point as the level of significance, all varieties were significantly injured. Canadian Thorpe and Glabron were least injured. All varieties were severely injured by the 4-320 concentration, so that this concentration was not of much value in showing varietal differences. Tentatively, however, one may place them in the following order: Canadian Thorpe and Glabron equal, Hannchen intermediate and O.A.C. 21 and Trebi most susceptible.

Although as has been suggested by Tisdale (32) varieties susceptible to seed injury should be treated with a weaker concentration of formaldehyde it does not necessarily follow that such a weakened concentration would be effective in controlling smut. It is preferrable that if formaldehyde be used we try to select varieties which are not susceptible to formaldehyde seed injury.

EFFECT OF FORMALDEHYDE TREATMENT OF WHEAT ON DIASTATIC ACTIVITY.

The following studies were made with the end in view of obtaining information on the nature of seed injury by formaldehyde. It seemed possible that a reduced activity of the enzyme in the seed might account for impaired germination caused by formaldehyde. Diastase was chosen for study since it is one of the most important enzymes concerned in germination.

Literature Review.

Kopaczewski (19) in 1912 could observe no effect of chloroform or toluene in a concentration of 0.1% upon the action of diastase, which action however, was prevented by 1.0% formaldehyde. Bokorny (3) in 1919 reported that maltase was injured by 0.1%, and destroyed by 1.0% formaldehyde. Sherman and Wayman (27) reported that formaldehyde concentration of 0.0035% and 0.035% distinctly depressed the activity of a number of amylases studied. They concluded that the loss of activity depends neither on the concentration of the enzyme or on the concentration of the substrate present, but directly and solely upon the concentration of the antiseptic. Atwood (1) concluded similarily that there is a reduction of the amount of starch digested with an increase of the concentration of the formaldehyde used.

Experimental Methods.

Diastatic activity as used in these studies was measured by the number of mgm. of maltose produced by 10 gm. of dry ground wheat in 1 hour at 27.5°C. The method used was Malloch's (21) modification of Rumsey's method (25). The procedure was as follows: the grain was treated with formaldehyde by immersing it in the solution for 2 minutes, following this it was air dried 36 hours and ground to pass a 1 mm. Wiley Mill sieve. Duplicate 10 gram samples were weighed into Kolrausch flasks and 100 cc. of Sorenson's HCl-Citrate buffer (pH 4.7) added. Digestion was carried out at 27.5°C. for exactly 60 minutes. Diastatic activity was calculated by subtracting the amount of naturally occurring reducing sugars from the amount determined after digestion.

Experimental Results.

The effect of the formaldehyde concentrations 1-400, 1-360, 1-320, 2-320 and 3-320 on the diastatic activity of seed of the wheat varieties Red Bobs, Marquis and Garnet was studied. These varieties as determined by field experiments represent respectively resistance, semi-resistance and susceptibility to formaldehyde seed injury. Results for the diastatic activity determinations are presented in Table XIV, and illustrated in Figure 12. Duplicate determinations checked very closely, and the figure presented represents the average of these determinations.

Table XIV.

Diastatic activity of wheat varieties treated with different concentrations of formaldehyde.

Formaldehyde	Diastatic activity.		
Concentration	Red Bobs	Marquis	Garnet.
Check	147.8	224.2	211.3
1-400	112.7	216.1	197.6
1-360	132.3	203.5	163.5
1-320	130.3	221.8	208.0
2-320	140.7	194.6	175.6
3-320	135.2	188.4	165.9

It is evident from the above table that formaldehyde decreases the activity of the diastase of the wheat kernel and that different concentrations have different effects on diastatic activity. Examining graphs plotted from these figures one at once notices the sharp drops in the curves at the 1-360 concentration in the case of Marquis and Garnet, and at 1-400 for Red Bobs. This would lead one to believe that the 1-360 concentration is more injurious to the enzyme than the 1-320 concentration in the case of Marquis and Garnet, and that 1-400 is the most injurious concentration for Red Bobs. Presumably the action of the chemical on the enzyme does not become manifest until penetration has taken place. Since treatment with formaldehyde was applied before the grain was ground, differences in permeability of seed coats may play a part in

Table XIV.

Diuretic activity of urea derivative showing the difference concentrations in formulations.

Formulations	Diuretic activity		
Concentration	Mean urine	Density	Remarks
Check	147.4	1.0	
1-400	134.7	1.0	
1-200	136.0	1.0	
1-100	134.3	1.0	
1-50	144.7	1.0	
1-25	153.3	1.0	

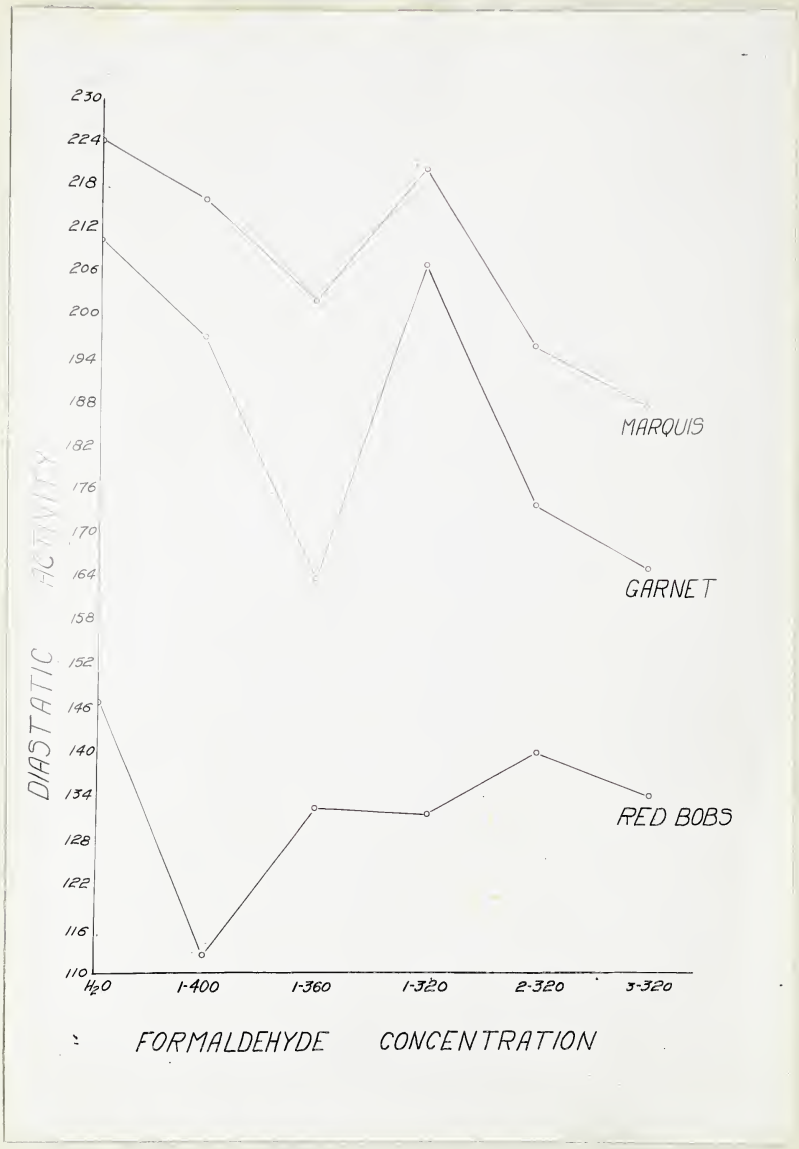

Figure 13.

Effect of seed treatment with various concentrations of formaldehyde on the diastatic activity of ground grain.

determining injury from different concentrations of formaldehyde. It is possible that certain concentrations of formaldehyde penetrate more readily and more rapidly than other concentrations.

Discussion.

Evidence accumulated from the above experiment points strongly to the fact that formaldehyde injury to seed occurs, partially at least, through its deleterious effect on the enzyme diastase. Diastatic activity of ground grain, following formaldehyde treatment, is shown to decrease with an increase in concentration of the formaldehyde used for treatment. It is to be noticed also that a 1-360 concentration of the formaldehyde has a very marked depressing effect on diastatic activity, and a 1-320 concentration has the least depressing effect in the varieties Marquis and Garnet.

In an earlier section of this paper it was shown that formaldehyde injury to wheat seed definitely increases the susceptibility of seedlings to attack by certain pathogenic fungi. It was also shown that treatment with a 1-360 concentration of formaldehyde resulted in excessive damage to wheat seedlings from several pathogenes studied, and that seedlings from seed treated with a 1-320 concentration of formaldehyde were least susceptible to damage from these organisms. Therefore it seems, that a decrease in diastatic activity is associated with a corresponding increase in severity of damage from pathogenic organisms. This relationship holds true in all cases for the concentrations 1-360 and 1-320, and for all the concentrations studied where soil-borne inocolum was used.

However, using seed-borne inoculum this is not so with the concentration 3-320 and may be explained as suggested above as due to the residual effect of the paraformaldehyde on the grain at this concentration.

In uninfested soil seed injury from formaldehyde increases progressively with an increase in concentration of the formaldehyde. In infested soil, however seed treated with a 1-360 concentration of formaldehyde consistently renders seedlings more susceptible to attack from pathogenic organisms than does a 1-320 concentration. Since the 1-360 concentration only behaves this way in infested soil it must in some way be associated with the pathogene, and one would be inclined to interpret the results as follows: On the one hand formaldehyde tends to cause seed injury and increase the damage from pathogenic organisms, and on the other hand it tends to possess fungicidal properties and reduce the damage from these organisms. Therefore at the concentration 1-360 one could say that its seed injuring properties were operating at a maximum to induce excessive damage from the organism. At the 1-320 concentration, where the least damage occurs from certain pathogenic organisms, one might say that the seed injuring properties of the formaldehyde were operating at a minimum and its fungicidal properties were at a maximum. With further increase in concentration of the formaldehyde the fungicidal effects tends to be masked by the seed injury effects which tend to increase disease rating in the case of soil-borne organisms. In the case of diseases where inocolum is seed-borne the fungicidal effect of the formaldehyde masks any predisposing effect and the result is a decrease in the severity of damage.

EFFECT OF FORMALDEHYDE ON DIASTATIC ACTIVITY OF DIFFERENT CEREAL VARIETIES.

Cereal varieties having exhibited definite differences in susceptibility to formaldehyde seed injury in the field, it was thought well to determine what effect different formaldehyde concentrations might have on the diastatic activity of these same varieties.

Experimental Methods.

Diastatic activity was determined as already outlined. Seed of the varieties of cereals to be tested was produced by the Cereal Division in rod row varietal trial plots in 1934. The varieties were therefore all grown and handled under similar conditions. Treatment of the grain was as follows: 60 grams of seed of the variety to be tested was immersed in a formaldehyde solution of the required concentration for 2 minutes, drained, covered 4 hours and then allowed to air dry for a period of 48 hours. The temperature of the formaldehyde solution and the drying room was kept as uniform as possible.

Experimental Results.

The first study had to do with the reaction of wheat varieties to the different concentrations of formaldehyde. The results are presented in Table XXIII and illustrated in Figure 14.

Table XXIII.

Diastatic activity of the ground grain of wheat varieties following treatment with different concentrations of formaldehyde.

Variety	Formaldehyde Concentration				Per-cent Decrease
	Check	2-320	4-320	8-320	at 8-320.
Red Bobs	172.4	170.6	165.4	149.1	13.5
Marquis	143.6	140.0	133.4	124.1	13.6
Reward	167.7	140.1	118.6	110.1	34.3
Garnet	179.8	128.7	109.8	87.6	51.3

The data in the above table shows that the decrease in diastatic activity from formaldehyde treatment varies markedly with the variety. This is brought out more clearly in Figure 14. The drop in the curve for Garnet is particularily interesting when compared for instance with the curve for Red Bobs where the drop is very slight, or even with the curve for Marquis. The differences are too large to be ignored and since other factors were constant it must be assumed that such differences are due to varietal reaction.

In experiments on the reaction of wheat varieties to formaldehyde it will be rembered that Red Bobs was most resistant to seed injury by formaldehyde and Garnet the most susceptible. It appears, therefore, that the reaction of wheat varieties to formaldehyde seed injury in the field is determined, at least partially, by the effect of the formaldehyde on the activity of the enzyme diastase. In this regard it is possible

Table XXIII.

Statistics relating to the normal limit of error, variable difference between the first and second determinations of temperature.

Variety	Number of Determinations	Probable Error	Mean Error	Standard Deviation
Red Rose	1754	1.05	1.40	1.75
Marquis	1880	1.00	1.34	1.67
Preston	1877	1.01	1.34	1.69
Garnet	1749	1.07	1.43	1.79

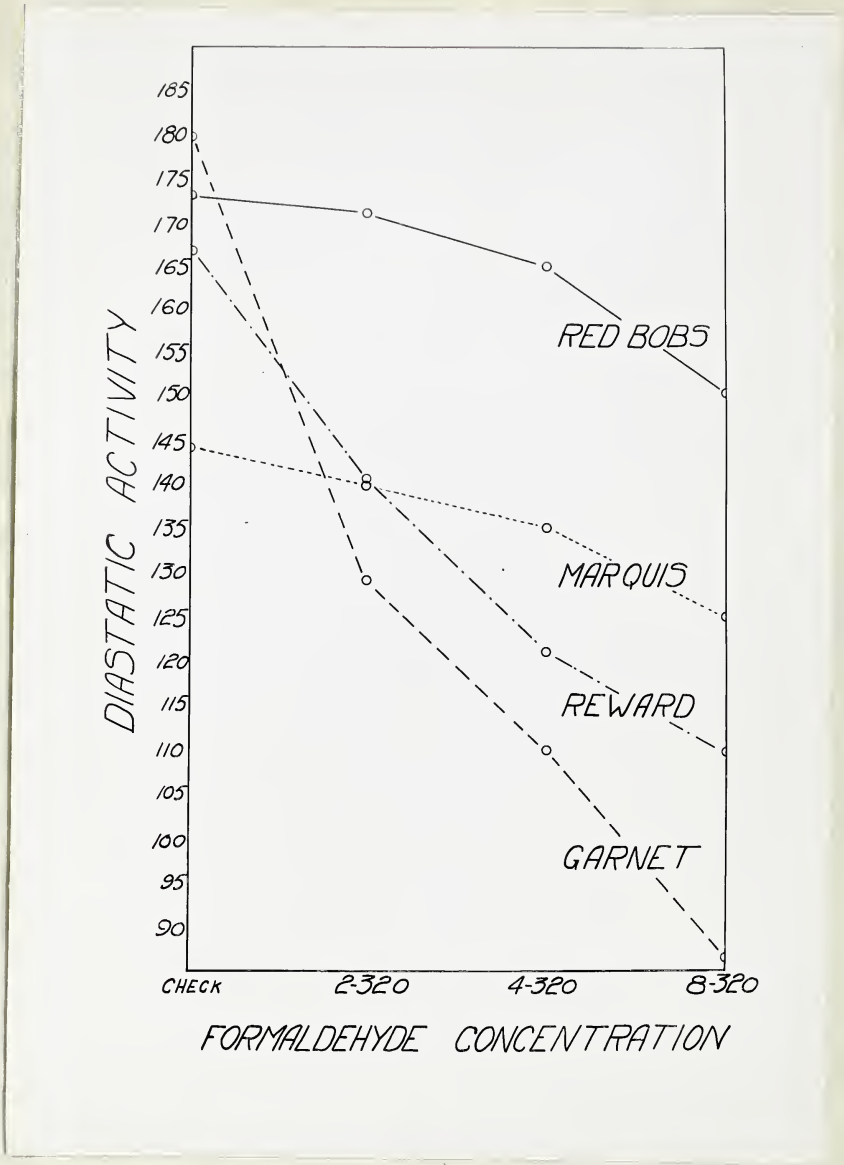

Figure 14.

Effect of formaldehyde seed treatment on the diastatic activity of ground grain of wheat varieties.

that the enzyme itself may differ in different varieties sufficiently to account for the injury to the seed of different varieties.

Since diastase is presumably borne within the tests in the embryo and in the endosperm another explanation of the varietal differences observed may be found in the relative permeability of their seed coats. It has been shown by various workers e.g. Atwood (1), Schroeder (26), Bakke and Plagge (2), and many others that the seed coat of wheat is permeable to formaldehyde, but it is quite possible that the different varieties vary in the permeability of their seed coats. An apparent difference in the seed coats of these varieties is their thickness. No literature is available on this point but it is recognized by millers that Reward has the thickest bran and Garnet has a very thin bran. Red Bobs and Marquis are intermediate. The thin bran in Garnet may account for the susceptibility of this variety to seed injury, but resistance to injury in Red Bobs may be governed by other factors. The permeability of the semi-permeable layer may not necessarily be correlated with the thickness of the bran, but varietal differences as demonstrated above may be due to differences in the nature of the semi-permeable membranes of different varieties, irrespective of bran thickness.

Atwood (1) makes the statement that formaldehyde retards the availability of carbohydrates to the germinating seedling, but does not inhibit the action of the diastase as digestion of the starch to sugar took place after the first three hours. There is no doubt a retardation as it has been demonstrated that seedlings from formaldehyde treated seed are invariably delayed in emergence from 1 to

3 days depending upon the concentration of the formaldehyde used. It is hard to conceive, however, that the action is only one of retardation and not inhibition when Garnet drops in diastatic activity from 179.8 to 87.6 units from the check to the 8-320 concentration of formaldehyde, particularly when the 8-320 concentration is sufficient to reduce emergence to 3 or 5%. These results would appear to justify the suggestion that there may be a complete inactivation of the enzymes which come in contact with the formaldehyde.

Results of formaldehyde on diastatic activity in varieties of oats and barley are presented in Table XXIV.

Table XXIV.

Diastatic activity of the ground grain of oat and barley varieties following treatment with formaldehyde.

	OATS				BARLEY		
Variety	Check	8-320	Per-cent decrease	Variety	Check	8-320	Per-cent decrease.
Victory	121.8	82.0	32.6	O.A.C., 21	123.3	119.3	3.2
Laurel	63.2	40.0	36.7	Can.Thorpe	154.1	130.2	15.5
Banner	134.4	84.8	36.7	Trebi	153.8	124.9	18.8
Gopher	122.8	54.0	56.0	Himalyan	182.8	132.2	27.7

It will be observed from the data in the above table that all varieties of oats are more reduced in diastatic activity by 8-320 formaldehyde than the barley varieties. This concentration of formaldehyde was used as a differential between the varieties. It will be observed, however, that great differences

do not occur between the oat varieties and possibly a weaker concentration could have been used to better advantage. It is interesting to note that the hulless variety Laurel shows no greater reduction in diastatic activity than Banner. Gopher in these tests was more injured than any of the other varieties. This corresponds to injury in the field where Gopher was more susceptible to formaldehyde seed injury than either Banner or Victory. In the barleys the hulless variety Himalyan is more injured than any of the hulled varieties. This shows the importance of the attached lemma and palea in protecting the seed from injury, but is somewhat contradictory to the finding in oats where the hulless variety Laurel is less injured than some of the hulled varieties. This indicates that factors other than the protective covering of the hull may be operating in reducing seed injury, and also that the nature of resistance in oats may be quite different from that in barley.

SUMMARY

1. Wheat is commonly sown in western Canada when soil temperatures are relatively low. It was found in these studies that maximum seed injury of wheat by formaldehyde occurs at soil temperatures around 15°C. This is approximately the temperature of the soil in the Edmonton district when much of the wheat is sown.

2. Wheat seedlings from formaldehyde treated seed were more severely attacked by certain pathogenic organisms than seedlings from water treated seed.

3. It was found that the severity of damage to

wheat seedlings caused by seed-borne inocolum of Helminthosporium sativum and Fusarium graminearum increased to a certain point with increasing concentration of formaldehyde used for seed treatment. However the residual effect of the formaldehyde concentration 3-320 on the seed reduced the severity of damage from these organisms.

4. The severity of damage caused by soil-borne inocolum of Pythium sp. and Ophiobolus graminis increased steadily as increasing concentrations of formaldehyde were used for seed treatment, and appeared unaffected by residues of the higher concentrations of formaldehyde on the seed.

5. With the exception of the bunt-fungi all the pathogenic organisms studied produced more damage to seedlings from seed treated with a 1-360 concentration of formaldehyde than to those from seed treated with a 1-320 concentration.

6. The 1-320 concentration of formaldehyde, the concentration usually recommended for treating cereal seeds, consistently produced least seed injury and tended to predispose the seedlings the least to attack by the pathogenes studied.

7. Wheat is most injured by formaldehyde seed treatment, barley the next and oats the least injured.

8. Varieties of each of the cereals wheat, oats and barley differ in their susceptibility to seed injury by formaldehyde. Red Bobs was the most resistant and Garnet the most susceptible of the wheat varieties studied. Leader was found to be the most resistant of the oat varieties and White Cross the most susceptible. Canadian Thorpe and Glabron

were most resistant of the barley varieties while Trebi was the most susceptible.

9. Formaldehyde treatment of the grain results in a decreased activity of the enzyme diastase within the kernel.

10. Diastatic activity of wheat, oats and barley grain following treatment with formaldehyde decreased with increasing concentration of the formaldehyde solutions used.

11. In Marquis and Garnet a 1-360 concentration of formaldehyde depressed diastatic activity more than a 1-320 concentration. In Red Bobs a 1-400 concentration was more injurious than either a 1-360 or 1-320 concentration.

12. The decrease in diastatic activity of the grain following treatment with formaldehyde varies with the variety used. The diastatic activity of the seed of Red Bobs was considerably less reduced than that of Garnet.

13. In the wheat varieties a decrease in diastatic activity of the grain following treatment with formaldehyde corresponds to the reaction of varieties to formaldehyde injury in the field.

14. In oat varieties a decrease in diastatic activity corresponds to injury in the field resulting from formaldehyde seed treatment. This did not hold for barley varieties.

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